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Art. XII.—*A Comparative Examination of the Blood of Certain Australian Animals.*

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(With Plates XVIII. and XIX.).

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The study of the histology of the blood is a comparatively recent one, since it appears only to have been taken up in earnest in the latter half of the 19th century. These early workers were necessarily hampered by imperfections in the apparatus at their disposal. Gulliver (1) in 1875 published the results of an exhaustive examination of the shapes and sizes of red corpuscles of vertebrates, and this had been preceded by a paper on the taxonomic import of the nucleus of these cells. He was followed in 1878 and '79 by the appearance of two publications by Erhlich, whose name in connection with histology and reactions of the blood is, of course, a household word to all students of the subject to-day. So lately as 1892, however, Newton Parker (2) writes: "The fact that the white corpuscles of the blood are not all alike is now well known in the case of most vertebrates, although it is not possible, in most cases at any rate, to state definitely whether these do or do not correspond to stages in the development of one and the same thing, and whether different functions are performed by these different kinds of leucocytes."

In later years the intimate relation between the state of the blood and various conditions of disease, together with the very perfect methods of manipulation which the modern knowledge of staining and fixing has evolved, have produced an extensive literature on the histology of the blood. The work done in this direction being principally descriptions of pathological conditions or comparisons of normal blood with that of diseased individuals, is mainly confined to an examination of man and the domesticated animals. In a recent paper (3) Drs. Cleland and Harvey Johnston have, however, endeavoured to point out that some indication of the probable line of evolution of vertebrate forms may be deduced from a comparative study of the shapes and sizes of the red cells of the blood. In the present paper I have essayed to collect some data in regard to the native Australian animals, and have tried to obtain as many types in each group as possible.

The chief difficulty has been the fact that the native animals available can seldom be said to exist under normal conditions, being principally captive specimens, mainly from the Zoological Gardens; and in many cases the smears were so distinctly pathological as to be useless for comparative purposes. Bearing this in mind I have been careful in forming generalisations from my results unless the material has been sufficient and reliable enough to warrant so doing. I have to acknowledge my indebtedness to Professor Spencer and Dr. Sweet, of the Biological School, Melbourne University, for the use of books, specimens, and apparatus; to Dr. Gilruth, sometime Professor of Veterinary Pathology; Acting-Professor MacDonald, Dr. Dodd, and Mr. H. R. Seddon—all of the Veterinary School, Melbourne University—for the use of books, smears, and for advice as to methods of staining, etc.; also to the members of the Biological Laboratory, past and present, especially to Mrs. J. L. F. Woodburn, for smears from wild and native forms of N.S.W.

Methods.

Actual Counts were only made in a few cases. The instrument used was the Thoma Zeiss Haemocytometer, with Hayem's diluting fluid. Where possible the blood was taken from the ventricle, immediately after death; and in most cases, for lack of time, the white corpuscles were counted with the red.

Smears.—Wherever possible the smears were fixed in alcohol before staining, and several specimens were obtained, so that at least two varieties of stains might be employed. Those stains found to give the most consistent results were those of Jenner and Giemsa ("Tabloid" brand). These, however, do not both react in the same way to the various classes of white cell, making the determination of a differential count somewhat uncertain at times. Thus most cells were found to differentiate better with Giemsa, and in some cases only with that stain, though Burnett (4) describes mast cells whose granules take a purple stain with Jenner. Mononuclear forms, especially of amphibia, showed best with Giemsa; while cells with eosinophile characteristics stained more satisfactorily with Jenner—with the doubtful exception of one marsupial form—and the crystalloid eosinophile cells showed their preference for this stain markedly. These observations are borne out by the statement of Daniels in "Laboratory Studies in Tropical Medicine." p. 67, where he remarks, in reference to slides stained with Giemsa. "They (eosinophile granules) do not form as conspicuous objects as specimens stained by Louis Jenner's stain.".

Nomenclature.

These facts have given rise to a difficulty in arriving at a completely satisfactory method of naming the various forms of leucocyte, and probably as Fantham (5) remarks, p. 726, "The differences in opinion of the various investigators are explicable by reference, to slight variations in the stains."

In arriving at any satisfactory nomenclature it has been necessary to compare the several methods employed by different investigators.

Erhlich, (6) dealing with human blood, distinguishes six normal types—1. Lymphocytes; 2. Large mononuclear leucocytes. Between 1 and 2 he states there are no transitional forms. 3. Transitional forms derived from 2. 4. "Polynuclear" leucocytes. 5. Eosinophil cells. 6. Mast cells. In addition he describes various pathological forms.

Burnett (4) distinguishes five varieties of leucocyte in normal blood—1. Lymphocytes. 2. Large mononuclear and intermediate forms between 1 and 2. 3. Polymorphonuclear forms, or finely granular oxyphils. In this group he includes those cells in the blood of birds which contain large spindle granules. 4. Eosinophiles. 5. Mast, which he describes as coarsely granular basophiles, but which I have usually found to be distinguished by their metachromatic staining properties. He also notes many degenerate forms of leucocyte, evidently present under fairly normal conditions, such as swollen or irregular nuclei; degenerating nuclei; ruptured cell bodies and pale nuclei, etc.; beside many forms found under pathological conditions, such as myelocytes, plasma cells, and various abnormal kinds of erythrocytes. The blood dust described by Burnett perhaps corresponds to the substance attributed by Cullen (7) to the free granules of the mast cells, and to which the same name is given.

Cullen (7) describes four kinds of leucocytes in fishes and birds. 1. Small mononuclear, which closely resemble the corresponding cells in man, and which I take to mean the lymphocytes of most classifications. 2. Large mononuclears. 3. Eosinophiles, in which he distinguishes a granular form, and an oxyphilic spindle form, in this respect differing from Burnett. From my own observations

I am inclined to agree with Cullen in placing the spindle granules of birds as eosinophile forms just as the spindle granules of the cat are described under that heading by Burnett. (*Goodall* (8) also remarks that neutrophile cells are absent in the fowl, their place being taken by eosinophiles, with oxyphilic spindles.) 4. Mast cells.

Fantham (5) distinguishes, in the normal blood of the grouse (a). Erythrocytes, among which he finds normal cells, cells without nuclei, and erythroblasts—cells which are rounder and have more spherical nuclei than the ordinary form, and whose cytoplasm stains blue with Giemsa.

(b) Leucocytes, under which he puts—1. Lymphocytes, both large and small, the large variety merging into small mononuclears.[1] 2. Large mononuclears, whose protoplasm is basophil, staining deeply with Giemsa, and less darkly with Jenner. 3. Polymorphonuclear leucocytes (Burnett), or crystalloid eosinophil cells (Cullen, Warthin). 4. Eosinophile leucocytes (Burnett) or coarsely granular eosinophile (oxyphile) cells. 5. Mast cells (coarsely granular basophile cells). 6. Thrombocytes, which suggest very narrow and slightly small erythrocytes. The whole cell is basophile in its reactions, staining rather faintly blue with Jenner's stain.

Gruner (14) gives a classification of the human blood cells based on biological principles, and, following this, a valuable summary of the work done on their comparative cytology, together with an exhaustive bibliography. Deriving all blood corpuscles from the primordial cell he describes various forms in both normal and pathological blood:—1. Red cells, which are only present in vertebrates, and may be divided into—(a) orthochromatic, (b) polychromatic, and (c) megaloblasts. Platelets are absent where the red cells are nucleated. Spindle cells occur in all vertebrates below mammals, and are pear-shaped, or almond-shaped plaques, which in birds are sometimes regarded as identical with the mammalian thrombocytes, and physiologically have the same function as the platelets. 2. Lymphocytes, uninuclear, basophile, nongranular cells, which may be divided into—(a) small, and (b) large lymphocytes, and (c) large mononuclear cells, with transitional forms between the two former and the latter, and characteristic of infra-mammalian species. 3. Large mononuclear cells, which are of two types—(a) lymphoid, and (b) granular, and which are present in all animals in which blood may be detected. They

[1] In this connection we must note Erhlich's statement that in human blood there are no transitional forms between lymphocytes and mononuclears. This raises the question as to the homology of these forms in the various groups of animal.

appear to play the part of macrophages in reptiles and amphibia, and are frequently possessed of so-called "secretory vacuoles." 4. Neutrophile leucocytes, consisting of an oval, large, basophile cell body, fibrillar in structure, with oxyphilic paraplasm; round, indented, or polymorphous nuclei; not found far back in the vertebrate scale, and probably not corresponding to the phagocytes of cold-blooded vertebrates. These apparently correspond to the polymorphonucleate cell of other writers, and are regarded by Gruner as being absent in their true specific form in cold-blooded vertebrates, the part of the human polymorph being played by the macrophage in the frog and reptiles. In birds he includes under this group mast cells, eosinophile cells, with rod-like granules (pseudo-eosinophiles), and cells of the same size full of minute oxyphilic granules. 5. Eosinophiles, with granules of various shapes and sizes. 6. Mast cells, which are mono- or polynucleate, variable in size, with a vacuolated cell body. They are divided into those containing fine, irregular granules, staining red-violet with Giemsa, and those containing scanty, coarse granules. Besides these there are forms more or less characteristic of certain pathological conditions, such as plasma cells, giant cells, etc.; as well as various structures which may represent stages in the life history of the normal blood cells. For the work under discussion I have adopted the following terms in an attempt to reduce the blood cells of the various groups of animals to a common classification.

1. *Erythrocytes.* Nucleated or non-nucleated cells, according to the group of animal. Normally staining yellow-orange or brick-red, but polychromatic or basophil forms were common in all species. In all cases irregular forms were present, even when the blood was not apparently pathological. In amphibia, reptiles, and some birds, I noted, besides these ordinary forms, structures which I have called spindle cells, and which I take to correspond to the thrombocytes of Burnett and Fantham. In the lower vertebrates these cells are thought to perform the function of blood platelets (Gruner). In many cases these cells were distinctly bi-polar, though in the majority they were drawn out at one end only. They were not as consistently basophil as the authors quoted describe, but their tendency seemed to be decidedly towards basic or polychromatic reactions. They have been described to me as artefacts, but as I have repeatedly observed them in the haemocytometer while making an estimation of corpuscles, and once in an examination of fresh frog's blood during a laboratory demonstration, while

I have frequently found them elongated at right angles to the length of the smear, I adhere to my first belief that they are fairly usual constituents of the blood of certain animals.

2. *Leucocytes.*

(a) *Lymphocytes.* Small, round or irregular cells, with basophil and practically homogeneous protoplasm. Deeply basophil nucleus filling the greater portion of the cell.

(b) *Mononuclear.* Larger basophil forms, frequently containing basophil granules, and with large and often excentric nuclei.

(c) *Polymorphonucleate.* Large forms, with irregular and frequently excentric basophil nucleus, the cell protoplasm often exhibiting faint acidophil properties, or even granules.

(d) *Transitional forms.* Basophil cells, whose nuclei present intermediate stages between (b) and (c).

(e) *Eosinophile*, containing large or small acidophil granules, and irregular nuclei.

(f) *Mast cells*, containing granules, staining more or less metachromatically, and faintly basophil cytoplasm.

Fishes. (Plate XVIII.; Figs. 1-6.)

Only one form was examined—the teleostean Sea-hedgehog (*Diodon histrix* (?))—and the smear contained many bacteria. The red cells were much rounder than those of batrachians or reptiles, averaging 12.5μ × 9.2μ. The protoplasm of many took a basic stain. One doubtful spindle form was observed, measuring 13.2μ × 8.3μ. The lymphocytes were round, and the most conspicuous leucocytes were mononuclear forms, with deeply basophil granules, averaging 12.4μ in diameter; while others resembling the polymorph type in general characteristics, but containing basophil protoplasm, averaged 11.6μ. No eosinophil forms were apparent on treatment with either Jenner's or Giemsa's stain. [1] Newton Parker (2) gives the average sizes of the red as much larger (40-46μ × 25-27μ), but Johnston and Cleland (3) remark that they find a wide diversity among fishes, dipnoi running as high as 29μ × 23μ, while in some teleosts their reading is as

1 Gruner (14) notes that in general the lymphocytes are fairly typical in this group, showing transitions to the large mononuclear type; also the fact that the mononuclear forms are typical of the lower vertebrates, occasionally replacing the polymorphs in function. He also draws attention to the absence of eosinophil and mast cells in fishes.

low as $6\mu \times 6\mu$. As these latter observers point out, the Teleostei have evidently branched off from the main stem, giving rise to batrachians and reptiles, so that there is little of comparative interest in this reading.

Batrachia. (Plate XVIII.; Figs. 7-17.)

Red Corpuscles.—Considerable variations in the size of all types of cell were observable in this group; but, with the doubtful exception of one slide from a tadpole, the young forms have larger corpuscles than the adult. Spindle cells were found in *Lymnodynastes dorsalis*, averaging $23\mu \times 12.45\mu$, also in fresh blood of *Hyla aurea*, used for laboratory demonstration purposes, and in the haemocytometer; while in the majority of cases basophil polychromatic reds were found. Some of these stained more deeply than others, and, with the exception of *L. dorsalis*, averaged smaller than the ordinary red. The chromatin of these forms did not stain as deeply as that of the typical erythrocytes. The smears also contained masses of a homogeneously staining substance, or, in other cases free nuclei (cf. Fantham (5) p. 728); while in one young *H. aurea* were forms with vacuolated protoplasm, and very densely stained nucleus. In some cases the red cells were apparently in a state of active division. This was observed chiefly in *L. dorsalis*. Gruner (p. 94) remarks seven varieties of red cell in the frog, giving their average size as $14.5 \times 25\,\mu$, and their number as half a million per c.cm, being much fewer than in other animals.

Lymphocytes varied much in size and shape, ranging from 6.6μ to $13.4\mu \times 5.3\mu$; and were occasionally, e.g., *H. aurea* and *L. dorsalis*, observed with the nucleus in a state of division. In *L. dorsalis* they showed a distinctly fringed outline, comparable to that described by Erhlich in human blood. They were always basophil.

Mononuclear forms were strongly basophil, and varied much in size, and also in relative numbers, showing all gradations from 8.6μ-19.9μ in diameter [cf. description of intermediate forms by Stephens and Christopher (9), p. 19], the average size falling, however, between 10μ and 14μ. The nucleus tended to become segmented, and the cell substance to contain granules.

Eosinophils were not, as a rule, clearly defined, with the exception of *L. dorsalis* and *H. peronii*, in which there was a decided increase in the relative numbers of these cells, together with a marked difference in the size of the granules as compared with those of other members of the group. The granules in these two forms closely resembled in appearance those figured by Burnett (4) for the horse. In *L. dorsalis* also, the eosinophil cells differed from those of other batrachia examined, in averaging larger than the true polymorph cells. Gruner, on the other hand, regards the eosinophil cells as forming the important features of the blood of amphibia.

Polymorph cells of the true type are denied by Gruner, but he notes forms containing a true polymorph nucleus, and faintly basophil or amphophil cell substance, and these I found were very distinct in most cases. In *H. peronii* these were represented by forms with somewhat irregular nuclei, much pressed to the side of the cell.

In tadpoles the leucocytes were apparently all of the mononuclear type, averaging 12.1μ-16.9μ; while the spindle cells and basophil reds containing large and less dense nuclei than the orthochromatic were numerous, and in one form the true red cells tended to become vacuolated. In such smears a differential count was obviously impossible.

Gruner regards the mononuclear as the primitive type of cell, and, as such, they are to be expected in the more or less undifferentiated blood of young animals.

BATRACIAN CORPUSCLES.

Name.	Percentage Counts of Leucocytes.			
	Lymphocytes.	Mononuclears.	Polymorphs.	Eosinophils.
Hyla aurea (young)	35.2 – 40.4	56.7 – 61.1	1.6 – 2.7	1.1 – .9
„ „ (adult)	51.2	28.3	8.2	1.4
Lymnodynastes dorsalis	68.2 – 73	10.6 – 12	15.7 – 13	3.2 – 2.1

BATRACIAN CORPUSCLES.

SIZES MEASURED IN μ.

Name.	Red. Spindle. L.	Red. Spindle. B.	Red. Ordinary. L.	Red. Ordinary. B.	Polychromatic and Basophil. L.	Polychromatic and Basophil. B.	Lymphocytes.		Mononuclears. Small.	Mononuclears. Medium.	Mononuclears. Large.	Polymorphs.	Eosinophils.
Hyla aurea (young			18.6	10.8	14	10.2	6.6	10.4 × 4.3	8.6	14.4 – 17.2	18.6 – 19.9	14.9 – 16.6	16.6 – 17.7
Hyla aurea (adult)			17.4	11.1	9.4		6.3	13.2 × 5.3	10.9	14.9		15.1	19.9
Hyla aurea (tadpole)			16.1	8.7								15.9	
Hyla peronii			15.5	11.2				10.95	10.7			12.1	11.6
Lymnodynastes dorsalis	23	12.45	20.4	11.6	22	11.1	6.6	10.8 × 6.1	10.8	14.1		13.7	12.45
Tadpole sp. (?)	18.2	9.1	18.2	10.6								12.1	
Lymnodynastes tasmaniensis (tadpole)			18.3	8.3									
L. tasmaniensis (adult)			16.1	9.4	15.93								

Reptiles. (Plates XVIII. and XIX.; Figs. 18-33.)

Red Corpuscles.—The counts of the absolute number of red cells varied, probably due to the effect of different seasons. For instance, *Chelodina longicollis* would not give enough blood for a haemocytometer count when pricked in May, apparently because the animal was then hibernating, and the estimation of its cells could not be made until it was killed in July.

The red corpuscles varied in size, the largest observed being found in *Chelodina longicollis*, and measuring 21.9μ × 13.3μ, which is a larger reading than that given by Cleland and Johnston (3), viz., 18.5μ – 19.5μ × 12.5μ. The increase in size in young forms as compared with adults of the same species characteristic of batrachia, is no longer apparent, except in *Tiliqua scincoides*, which ranked next in size to *Chelodina longicollis;* but for purposes of comparison I was not, in this case, able to obtain the adult. My measurement (19μ × 10.3μ) is, however, a slightly larger reading than is given by Cleland and Johnston for the same form (presumably adult). In one case, *Til. nigra-lutea*, the cells varied enormously, running from 19.9μ × 11 6μ to 9.9μ × 6.6μ; and in *Gramatophora barbata* there were some small round cells, appearing normal in reaction to stain (microcytes), while in *Trachydosaurus rugosus* these small forms were also observed, bearing in this case a very darkly staining nucleus. Anaplasms were common in the cell of *Chel. longicollis*, while others showed different stages of vacuolation, and variously disintegrated structures, closely resembling those of *Hyla aurea* were found. In other cases the red cells seemed to be losing their nuclei, e.g., in *Tiliqua scincoides* (10). In some the nuclei of the ordinary forms tended to become irregular, with a distinct appearance of budding, and in the young specimen of *Gramatophora muricata* one was observed in a state of division. The reaction to stain was fairly normal, but in some the cytoplasm of ordinary red cells took on a green tinge with Jenner.

In reptiles, in distinction to batrachia, the larger forms of leucocyte approach more nearly the size of erythrocytes, the largest observed being the eosinophil cells of *Chel longic.*, which measured 21.5μ. Spindle cells were very common in all specimens, and might be pointed out at one or both ends. They rather inclined towards basophil characteristics, and among such cells binucleate forms were fairly numerous, as well as among the ordinary spindles. In the young *Tiliqua scincoides*, in which these cells were first observed, they averaged slightly smaller than the ordinary erythrocytes, with rounder nuclei. The smaller cells tended to be basophil, the larger staining normally. Normal basophil cells were also common, their nuclei being larger, and taking the stain less darkly than the ordinary forms, and showing a great tendency to branch or bud; in fact, in *T. rugosus* one was observed showing three nuclear masses.

Lymphocytes varied in size, the largest being found in young *Gramat. muricata*. These were distinctly oval in shape, measuring $11.9\mu \times 6.6\mu$. On the other hand those of young *Tiliqua scincoides* ran smaller than any other form, viz., 4.8μ. The percentage counts also varied, being fairly high in young individuals. The fringe of ragged protoplasm observed in some batrachia was also seen in this group (*Trach. rugosus* and *Til. nigra-lutea*). In the former these cells were most distinct when treated with Jenner's stain, and an appearance was obtained resembling division. The general shape varies from oval in *Gram. muricata* to round in *Trach. rugosus*, while in other cases it was almost impossible to distinguish lymphocytes from free nuclei.

Mast cells were common in this group, averaging larger than the mononuclear forms, but they were not present to any large percentage. The greatest number was found in the young of *Gram. barbata*, where they were present to the extent of 13.8 per cent. of the total white cells. They were common in *Chel. longicollis*, in which, as in *Trach. rugosus*, they showed both large and small varieties. In *Trach. rugosus* also, one was seen apparently in a state of division, and in this species the granules of the corpuscle were distinctly divided into large and small; while some cells appeared transitional in staining between the mononuclear and the mast variety, staining more darkly purple than the ordinary mononuclear forms, and with a few characteristic granules in the protoplasm.

Transitional forms of ordinary type were not observed.

Mononuclear cells varied in size, and might be divided into two classes—(a) Small, ranging from 7.9μ-9.9μ; (b) those corresponding more nearly to those of other forms, and ranging from 11.7μ-15.4μ, while in the carpet snake (specimen in very pathological condition) they ran up as high as 21.1μ.

The percentage counts were fairly high, but it was difficult in many cases to make a rigid distinction between large specimens of class (a) and small specimens of class (b). The small forms of *Chel. longicollis* were distinguished from the lymphocytes by the presence of many basophil granules. In others (*Gram. barbata*) the protoplasm tended to be vacuolated, and in *Til. scincoides* this vacuolation extended to the nucleus (cf. Erhlich, p. 86). As in amphibia Gruner regards these cells as playing the part of macrophages, true polymorphs being absent.

Polymorphonucleate Cells.—These were roughly about the same size as the eosinophiles, but gave a small percentage count except in young *Gramat. muricata*, where they ran up to 42 per cent. of total leucocytes; while in the Monitor it was not possible to distinguish them from the eosinophil cells. In smears from *Chel. longicollis*, large cells were seen in which the nucleus was pressed to one side, and the protoplasm scarcely stained at all. In other cases, e.g., *Gram. barbata* forms containing a horseshoe-shaped nucleus, and faintly pink protoplasm, scarcely to be distinguished from eosinophils, and comparable to those of some frogs, were seen. Similar cells were noted in *Til. nigra-lutea*. In other cases, e.g. *Gram. muricata*, the protoplasm was vacuolated, and the nucleus not so strongly basophil, as in the ordinary types. These, under Gruner's classification, must be regarded as eosinophils

Eosinophil Cells.—These were fairly well marked, though giving small percentage counts, except in *Chel. longicollis*, in which the granules could be distinctly divided into small and large. The latter closely resembled the spindle-shaped structures of birds, and were much more numerous than the former, the total eosinophil count giving 40 per cent. of leucocytes present. In size, with the exception of *Chel. longicollis*, which ran as high as 21.5μ, these cells average about the same as the polymorph forms. The eosinophils of *Chel. longicollis* are further peculiar in showing, besides the spindle-shaped granules already mentioned, finely and coarsely granular cells, the latter not unlike those of *Lymnodynastes dorsalis*, among the amphibia, though not staining so distinctly. In most cases, also, in this species, the eosinophil granules, with the exception of the large ones, did not stain with Jenner at all, so making it hard to distinguish true polymorphs from eosinophils, both of which carry nuclei pressed to the side. The same fact was also observed in *Gram. barbata*. In *Til. nigra-lutea* these cells did not show up with either Jenner or Giemsa; while *Trach. rugosus*, both young and adult, showed scattered granules staining best with Jenner, some cells being not clearly granular, while all had the nucleus pressed to one side. On the other hand, in *Til. scincoides* the nucleus was round in distinction to that of the polymorph cells, and the granules were few and refractive. Gruner questions the analogy between the cells containing spindle-shaped granules (crystalloids), and the eosinophil cells of mammals (human), just as in places the "pseudo-eosinophile" cells of birds in the polymorph series. If the structures I have called polymorphs in amphibia and reptiles, and which show amphophil or acidophil affinities, are to be regarded as eosinophils, then this class of cell is certainly the most characteristic of amphibian and reptilian blood.

REPTILIAN CORPUSCLES.

Names	Reds. per c.mm.	Whites. per c.mm. (taken with red)	Leucocytes %				
			Lymphocytes.	Mast.	Mononuclears.	Polymorphs.	Eosinophils.
Gramatophora barbata	1,589,416	50,000	14.5		65	12.2	8 03
Gramatophora muricata (young)			37.9	13.8		42.5	
Tiliqua nigra-lutea	988,095	21,875					
Trachydosaurus rugosus	753,125	12,500					
T. rugosus	1,222,222	33,333	28.3	3.5	51.1	16.2	.6
T. rugosus	1,522,222	43,333	55.0	2.4	40.0		
T. rugosus (young)	646,423	32,812	42.0	4.2	40.0	19.5	1.4
Tiliqua scincoides (young)	721,428	15, 33	65.6		30.6	1.8	2.8
Chelodina longicollis	102,380 (taken in Winter)	7,386 (taken in Winter)	26.1	1.5	23.7	8.4	40.0
Varanus (varius or gouldi)			47.9		29.9	22.3 [Polymorphs and Eosinophils together]	

REPTILIAN CORPUSCLES.

SIZES MEASURED IN μ.

Name.	Red. Spindle. L.	Red. Spindle. B.	Red. Ordinary. L.	Red. Ordinary. B.	Polychromatic and Basophil. L.	Polychromatic and Basophil. B.	Lymphocytes.	Mast.	Mononuclears. Small.	Mononuclears. Large.	Poly-morphs.	Eosino-phils.
Gramatophora muricata (young)			16.7	11.6	11.1		11.9 × 6.6	14.6		9	15.6	
G. barbata	15.7	7.5	16	8.96			6.6		7.9	14.5		15.27
Tiliqua nigra-lutea	15.9	8.3	17.9	10.2	16.6	11.4	6.4	11.6	1.3	13 2		16.6
Trachydosaurus rugosus	18.9	11.1	16.4	9.6	16.2	10.9	5.9	10.9		12.9		14.9
" " "			15.7	8.79	14.9	9.46	6.4 × 8 6	14.1 × 8.3	9.96	11.78 × 15.77	12.6	12.4
" " " (young)	16.6	8.7	16.8	10.67	18.2	11.6	7.5 × 4.2	11.6	7.6	12	13.2	
Tiliqua scinoides (young)	17.	6.6	19	10.3	13		4.8			15.4		16.7
Chelodina longicollis			21.9	13			7.3	16.6 × 9.1				21.5
Varanus (varius or gouldi) or Monitor			16.9	9.6	16.2	10.9	6.97	9.96	10.2		14.27	11.28
Carpet snake			17.9	10.37			5.39		10.2	15.6		14.7
									(8.3 – 12.45)	(13.2 – 21.1)		

Aves. (Plate XIX.; Figs. 34-37.)

Red Corpuscles.—The actual counts varied, but scarcely beyond the limits of the figures given by Burnett (4) for the blood of the domestic fowl, the highest being found in the Black Mountain Duck, and the lowest in the White Pekin. In size they averaged smaller than those of reptiles, the largest being found in the Spoonbill ($16.1\mu \times 8.7\mu$). The cells of young individuals showed an increase in size as compared with those of the adult. Basophil types (thrombocytes or erythroblasts of Fantham?) were not common, but were observed in the Chestnut Breasted Teal and young Mudlark and Heron, and averaged smaller than the ordinary forms. In the Heron a few cells resembling the spindle forms of reptiles were seen, but this smear was full of cocci, and in all probability not normal. In the Spoonbill, young Mudlark, and young Mountain Duck the basophil cells were also well marked, and showed great variety of shape, some even approaching that of spindle cells. It is worthy of note, from the standpoint of evolution, and in view of the occurrence of these cells in amphibia and reptiles, that they were more conspicuous in the younger forms of the birds examined, which is to be expected if they are a primitive type. Gruner, however, states they are present in all vertebrates below mammals. In the Spoonbill and young Mudlark also, the nuclei of many red cells were slightly moniliform, and in some cases almost completely divided, with the most dense chromatin at the centre of the nucleus. This aggregation of chromatin was best marked in the basophil forms, in which also division of the nucleus was well seen. Degenerate cells, resembling those found in reptiles, and described by Fantham (5), and consisting apparently of free nuclei, non-nucleated fragments and nuclei surrounded by a thin film of protoplasm, were also observed.

Lymphocytes.—These varied in size from 3.8μ in the Black Mountain Duck (*Anas superciliosa*), to 6.7μ in the Spoonbill. Their percentage counts were fairly high, running to 75.8 per cent. of the total leucocytes in the Chestnut Breasted Teal. In the young Mudlark the count was extraordinarily low (15.8 per cent.). These cells have a decided tendency to aggregate (cf Fantham (5)); and degenerating forms appearing like large nuclei of lymphocytes were present in certain smears, being in many cases difficult to distinguish from the true cell (cf. Burnett (4)). In the young Mudlark they are surrounded by a clear ring of cytoplasm.

Mast Cells.—These were not found on any slide, with the possible exception of the Chestnut Breasted Teal (*Casarca tador-*

noides) smear stained with Giemsa. In the Heron and Spoonbill there were also doubtful forms, but neither of these slides were very reliable, being fairly pathological.

Mononuclear Cells.—These varied in percentage count, but never ran higher than 28 per cent. of total leucocytes. They were distinctly divisible into small and large types, the former ranging from 6.8μ-9μ, and the latter from 8.5μ-14.5μ. The relative difference between the two forms ran as high as 6.5μ in one bird (Heron).

Polymorphonucleate Cells.—These were difficult to distinguish from the eosinophil, and a percentage count could only be made in the young Mudlark, in which they amounted to 3.5 per cent. of the total leucocytes. In this species also, their diameter was only 6.2μ, while that of the true eosinophils ran as high as 11.6μ. Gruner includes under this head mast cells, eosinophiles with rod-like granules, and cells of the same size full of minute oxyphile granules.

Eosinophil Cells.—In general, the size of these cells averaged less than those of reptiles. They were in most cases divided into those with round granules, and those with the spindle or crystalloid variety (polymorphonucleate leucocytes of Burnett, neutrophile of Gruner), though it was not always possible to distinguish clearly enough between the two forms in order to make a differential count. The crystalloid variety were particularly well marked in the White Pekin Duck and young Mudlark, in each of which they gave a high percentage count, very greatly in excess of the ordinary cells with rounder granules. As a rule the percentage of eosinophil corpuscles was fairly high, running up to 32.4 per cent. of total leucocytes in the Black Mountain Duck. In some cases, notably the Heron, the granules were very sparse, resembling those of certain reptiles, while in the Black Mountain Duck several large mononuclear cells showed an apparent eosinophilous granulation. In the Ibis there were distinctly three forms, (a) with small granules, (b) with ordinary spindles, (c) with large spindles—the last two classes being about equal in number, and varying slightly in size (11.6μ-10.9μ).

AVIAN CORPUSCLES.

Name.	Reds.	Whites.	Lymphocytes.	Mononuclears.	Polymorphs.	Eosinophils.
	per c.mm.	per c.mm.				%
Muscovy Duck	2,350,000	91,600	67.0	5.06		28.0
Asarca tadornoides (chestnut-breasted teal)	3,000,000	55,555	75.0	5.35		18.4
Anas superciliosa (black mountain duck)	4,450,000	50,000	64.9	6.5		32.4
Ardea (Sp. ?) (heron)			56.4	27.9		15.6
Spoonbill			69.6	1.35		29.0
Ibis			65.9	25.5		8.5
Grallina picata (young mudlark)	3,768,750	103,125	15.8	28.0	3.5	53.5

AVIAN CORPUSCLES.

SIZES MEASURED IN μ.

Name.	Red. Ordinary. L.	Red. Ordinary. B.	Polychromatic and Basophil. L.	Polychromatic and Basophil. B.	Lymphocytes.	Mononuclear. Small.	Mononuclear. Large.	Polymorphs.	Eiosnophils.
Muscovy Duck	10.6	6.6			4.2		7.4		9
Asarca tadornoides (chestnut-breasted teal)	10.8	5.7	8.2		5.4	6.8	11		8.9–11
Anas superciliiosa (black mountain duck)	10.6	6			3.8		8.5		8.7
Anas superciliosa (young)	13.9	7.4			-	-	-	-	-
Ardea sp (?) (heron)	13	8.5			5	8	14.5		12
Spoonbill	16.1	8.7			6.7				13.2
Ibis					6	9	13.6		10–11
Grallina picata (young mudlark)	12.6	7.3	10.9	8.3	6.6	7.4	11.6	6.2	11.6–10.6

Mammalia. (Plate XIX. ; Figs. 38-44.)

The representatives examined in this group all belonged to the Metatheria, being either marsupials or monotremes.

Platelets were observed for the first time in this group, being absent in all forms with nucleated red corpuscles (Gruner); and were very common in all species. In some of the marsupials they appeared to have a definite outline. Schäfer (12), p. 47, mentions blood platelets or thrombocytes in the frog, but I have found nothing to correspond strictly with these cells in appearance outside the mammalia.

Red Corpuscles.—These approached very nearly the actual number per c.mm in human blood. There was a marked decrease in the size of the cells as compared with in vertebrates; and in all cases they were non-nucleated, bi-concave discs. The variation in diameter is also much less than in other groups (5.1μ-7.8μ). In the young platypus (*O. anatinus*) they showed the greatest range, running from 6.6μ-9.9μ. There seemed to be no definite relation between the age of the animals and the size of the corpuscles. Poikilocytosis was fairly common in many marsupials, as well as polychromatophilia, while normoblasts were frequently present.[1]

There were also, in the smears of marsupial blood, a large number of corpuscles of normal shape, but small size (microcytes).

Lymphocytes averaged very much larger than the corresponding cells in birds, the greatest diameter being found in one species of wombat (*Phascolomys*), but there was no marked tendency towards increase in size in the young. The percentage counts varied enormously, running as high as 72.6 per cent. of the total leucocytes in *Trichosurus vulpecula;* but I could find no definite relationship between age and numbers, though, as a general rule, the young forms showed a high percentage count. In *T. vulpecula* and others the nuclei of some lymphocytes appeared to be distinctly dividing, and the size and amount of protoplasm in relation to the nucleus varied, but apparently the lymphocytes were non-granular, thus differing from the mononuclear forms. In *Petaurus breviceps* the lymphocytes were divided into two classes—(a) small, with very distinct outline to the nucleus, and very little cell substance; (b) larger cells, with less clearly marked nuclei, and protoplasm barely showing (cf. Gruner). In the young echidna the lymphocytes varied much in size, and were very granular; while the nuclei of several in the wombat smears showed distinct lobing, giving a kidney-shaped appearance. (Transitional forms?).

Mast cells were very rare, being only observed in *T. vulpecula* and the wombat, and in neither case numbering as much as 1 per cent. of the total leucocytes.

Mononuclear cells were divisible in some cases into small and large, and averaged as a rule, larger than the polymorphs[2] They showed, particularly in the young platypus, a great variation, as

1 Cleland and Johnston (3) suggest this may be an archaic feature.

2 Stephens and Christopher (9) remark on the frequent occurrence of "intermediate leucocytes," mid-way between the large and small mononuclear cells.

well as an increase, in size (12.8μ-18.1μ). Percentage counts were never very large, reaching the highest in *P. breviceps* (female with young), where these cells numbered 35.7 per cent. of the total leucocytes. Degenerating cells (cf. Burnett) were common, while in others the nucleus tended to become vacuolated (cf. Erhlich (6), and one cell of *T. vulpecula* seemed to be clearly dividing. The chromatin of the nuclei in many showed very distinctly.

Transitional forms, between the mononuclears and the polymorphs, were observed in four cases, and in size corresponded most nearly with the polymorphs, but in no case did they amount to 3 per cent. of the total leucocytes.

Polymorphonuclear cells were numerous, reaching as high as 76 per cent. of the total leucocytes in the female wombat. In size they approximated, on an average, to the eosinophils, being particularly small in one species of *Echidna histrix*, in which the cytoplasm stained very indistinctly. In *T. vulpecula*, on the other hand, they showed fine acidophil granulations (cf. Schäfer (11) p. 34).

Eosinophil cells never gave a high percentage count, falling particularly low in echidna, in which, neither in the young nor adult form, did they reach 1 per cent. of the total leucocytes. They seemed to average slightly larger than the polymorphs, though this was not an absolute rule. The granules of some were very scattered, while in others they were very fine, resembling the finely granular polymorphs of some forms. In the young platypus they were only distinguishable with Giemsa's stain. Erhlich (6), p. 179, notes among the a-typical forms of white corpuscles which may be present, dwarf forms of the eosinophil variety, and I found that these were also very common in many marsupials. Macrophages were also present in the form of large basophil mononuclear cells, containing partially disintegrated corpuscles of various types.

MAMMALIAN CORPUSCLES.

Name.	Red. per c.mm.	White. per c.mm.	Lymphocytes. %	Transitional forms. %	Mast. %	Mononuclear. %	Polymorphs. %	Eosinophils. %
Echidna histrix			12.5			18(+transitional)	68.2	.77
,, ,, (young)	6,520,000	60,000	43.6			7.5	48.5	.3
Ornith. anatinus (young)	7,671,428	17,187(?)	40.7	2.8		2.8	47.	4.
,, ,, ,,	6,682,142	21,875						
Petaurus breviceps (young)			37.7	3.9		10.4 (+transitional)	45.4	2.6
,, ,, (mother)			59 – 66			35—23	4.2 – 7.4	2.4
Trichosurus vulpecula			27.	1.5		5.28	64.7	1.3
,, ,,			72.6		.83	9	16.3	1.2
Pseudochirus peregrinus			16.8			19.9 (+transitional)	60.9	.78
Phascolomys wombat (male)			50,1			5.8	37.0	6.4
,, ,, ,,			18.4			4.7	76.8	
,, ,, (female)			50.7			3.4	43.6	2.7
,, ,, ,, (half-grown)			61.8			3.4	32.2	2.4
,, ,, ,, (full-grown			50.7			2.6	41.8	4.9
,, ,, (female)			39		.4	2.09	57.2	1.2
,, ,, (male)			49			2.4	46.7	1.6
,, ,, ,,			45			2	45	8.6

MAMMALIAN CORPUSCLES.

SIZES MEASURED IN μ.

Name.	Red.	Lympho-cytes.	Mast.	Mononuclears Small.	Mononuclears Large.	Transitional forms.	Polymorphs	Eosinophils.
Echidna histrix	6.6	9.6		8	11.6	6.6 – 9.1		
,, ,, (young)	6.5	10.3			15	13		14.9
Ornithorhyncus anatinus	5.1							
,, ,, (young)	7.8 (6.6 – 9.9)	10.2		12.86 – 18.1 (11.6 – 14.9)			10.2	10.45
Trichosurus vulpecula	6.2	9.3		13.6		13.9	12.3	14.3
Petaurus breviceps (young)	5.7	9.3		15.3		12.4	13.1	15.1
,, ,, (mother)	6.2	9.4		15.5		13.9	13.6	
Phascolomys wombat (male)	5.9	11.2		11.7			10.7	11.4
,, ,, (male)	6.1	10.1		13.2			11.1	
,, ,, (female)	6.6	10.6		14.9			12.4	10.89
,, ,, ,, (half-grown)	6.4	10.29					11.95	10.37
,, ,, ,, (full-grown)	6.7	10.1					11.6	10.9
,, ,, ,,	6.4	10.6	11.6 × 14.9	13.7			11 7	13.28
,, ,, (male)	6.8	11.4		15.3			11.7	13.7
,, ,, ,,	6.6	10.79		9.2 × 20.75			12.7	13.58

Summary.

Red Corpuscles.—Miss Clay-Pole (12) notes the decrease in size of red cells in passing from generalised to specialised types, and associates this with an increased haemoglobin capacity. I have found the same fact borne out by my observations, as well as a corresponding increase in the actual number of red cells, which, by increasing the total surface of the red cells, augments the area over which oxygen may be absorbed. There was a tendency towards larger size in young forms, which was, however, hardly apparent in marsupials. Spindle cells, when present, tended to be polychromatic or basophil in reaction, and were only found in any number in amphibia and reptiles.

Leucocytes.—(a) *Lymphocytes*, as a general rule, seem to be more numerous in young forms. The size was also greater in the young, and increased in passing through the various groups, being largest in marsupials. This large size in the young is evidently a reversion to the primitive type.

(b) *Mast cells* seemed to be more characteristic of the lower groups, as they were observed only once in marsupials, though they were numerous in reptiles.

(c) *Mononuclears* were more numerous in young animals, and the only form of white corpuscle found in the tadpole was nearest this type—primitive, according to Gruner. The average number of these cells became much less in monotremes and marsupials. In amphibia there were three distinct classes, according to the sizes, a giant type being well marked in the young. In reptiles only two classes were apparent, and the average size in the adults was less than in amphibia; while in birds they were smaller again, but still of two kinds. Monotremes resembled reptiles; but in marsupials we find one size with no marked difference between the young and adult.

(d) *Polymorphonuclears* in amphibia were not easy to distinguish from mononuclears by staining, and were also few in number; both facts being more clearly marked in the young. In reptiles also they were not clearly differentiated, and were only distinct in one bird. In monotremes, and still more in marsupials, they were numerous and apparent, except in the case of the adult female *Petaurus breviceps*, which was suckling its young when the smear was taken. Perhaps this fact may account for the extraordinary decrease in polymorphs, and rise in number of mononuclears in this particular specimen.

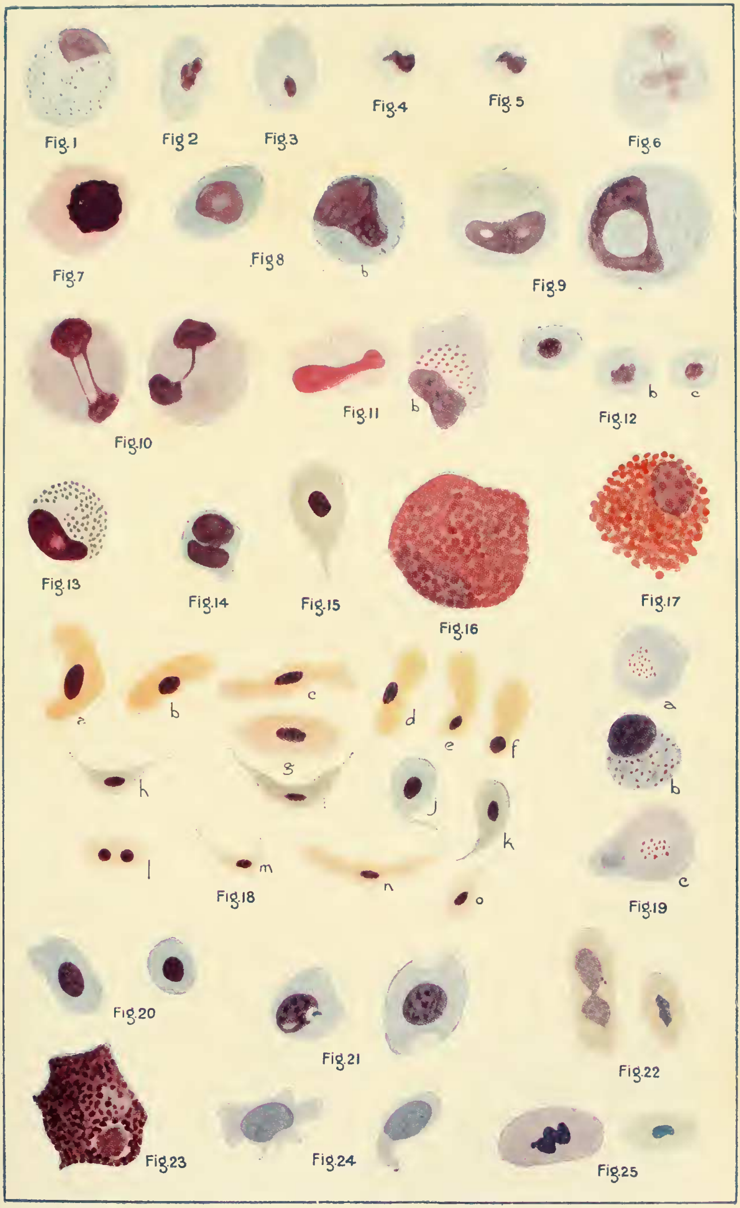
Fig. 1
Fig 2
Fig.3
Fig.4
Fig. 5
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Fig.44